## Remarks

The Office Action mailed August 13, 2002 has been received and reviewed. Claims 1-17 having been canceled, claims 18-20 having been amended, and claims 21-43 having been added, the pending claims are claims 18-43.

Support for the new and amended claims is found throughout the specification. For example, support for amended claims 18-20 is found in original claims 17-20 and support for new claims 21-34 is found in original claims 1-14. Support for new claims 35-37 is found on page 3, lines 13-18, page 2, lines 14-15, and page 11, line 11 of the specification; support for new claims 38-40 is found on page 13, line 18 and page 14, lines 8-12 of the specification; and support for new claim 41-43 is found in original claim 1 and on page 13, line 18 and page 14, lines 8-12 of the specification. Claim amendments have been made to clarify the claimed invention and do not narrow the claimed invention. No new matter has been added.

Reconsideration and withdrawal of the rejections are respectfully requested.

## Examiner Interview

An Examiner's Interview was held on October 8, 2002 between the Applicants' Representative and Examiner Woitach. The claim rejections of record were discussed. The Examiner is thanked for the courtesy of this telephonic interview.

**Election/Restriction**

The Examiner is thanked for the reconsideration and withdrawal of the Restriction Requirement between Groups I and II.

**Objection to the Specification**

The Examiner objected to the specification because page 7, lines 1 and 24 and page 8, line 1 of the disclosure contain embedded hyperlinks and/or other forms of browser-executable code. This objection is respectfully traversed. MPEP §608 - "Hyperlinks and Other Forms of Browser-Executable Code in the Specification," states "that hyperlinks and other forms of browser-executable code . . . are not [to be] included in a patent application." As explained in the Examiner Note section of MPEP §608, "[e]xamples of a hyperlink or a browser-executable code are a URL placed between these symbols '< >' and a http:// followed by the URL address." Pages 7 and 8 of the specification recite "nih.gov/news/stemcell/primer.htm." This recitation lacks both the "< >" and the "http://" designations, and thus, does not qualify as a hyperlink or a browser-executable code according to the definition provided in MPEP §608. Thus, it is respectfully submitted that the information presented on pages 7 and 8 of the specification is in proper format.

**Objection to the Claims**

The Examiner objected to claims 1-20, stating that although an election of species was made to a degenerative disorder, the claims broadly encompass any disorder. The Examiner

requested that the claims be amended to reflect the elected invention. This is respectfully traversed. First, according to MPEP § 809.02(a), Applicants' claims will be limited to a single, elected species only if no generic claim is finally held allowable. Thus, it is inappropriate, at this time, to require amendment of the claims, including generic claims, to limit the claims to the elected species only. Further, it is noted that the election of species requirement, mailed May 1, 2002, extended only to claims 1-16. Claims 1-17 have been cancelled. Thus, the objection to claims 1-17 is moot. Claims 18-20 were not included in the election of species requirement. Thus, the objection to claims 18-20 is incorrect. Withdrawal of this objection of the claims is respectfully requested.

**The 35 U.S.C. §112, First Paragraph, Rejection**

The Examiner rejected claims 1-20 under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. This rejection is respectfully traversed.

First, it is respectfully submitted that this rejection of claims 1-17 is rendered moot in view of the cancellation of claims 1-17.

*Amended Claims 18-20*

Claims 18-20 are drawn to a method of producing enhanced levels of insulin in a subject comprising implanting neural stem cells and/or progeny thereof into the pancreas of the subject.

The Examiner stated that the "breadth of the claims is large, encompassing any type of stem cell, any means of delivery and affecting treatment for any type of disorder," and that the specification provides insufficient guidance to teach how to engineer the delivery of insulin to any therapeutic effect" (see pages 8-9 of the Office Action mailed August 8, 2002). Applicants respectfully disagree with this statement; however, the rejection is rendered moot in viwe of the amendments presented herein.

Claims 18-20 are drawn to neural stem cells; the delivery of these neural stem cells to the pancreas by implantation; to achieve an enhanced production of insulin in the pancreas. Thus, the claims do not encompass any type of stem cell, any means of delivery, for affecting treatment for any type of disorder. Applicants submit that the specification presents adequate guidance to make and use the claimed invention with a reasonable expectation of success and without undue experimentation.

Neural stem cells (NSC) are capable of differentiating not only into cells of neuronal and glial lineage (see page 6, lines 15-17 of the specification ), but are also capable of differentiating into cells of hematopoetic and skeletal muscle lineages (see Micci et al., *Gastroenterology* 121:757, 2001). Furthermore, NSC from the adult mouse brain can contribute to the formation of tissues such as liver, stomach, and intestine in chimeric chick/mouse embryos (see Micci et al. p. 757). Thus, NSCs have a broad developmental capacity and can give rise to a multitute of cell types upon transplantation into differentiated tissues. Other types of stem cells, such as pancreatic stem cells (see Cornelius et al., *Horm. Metab. Res.* 29:271, 1997) and adult hepatic stem cells (see Yang et al., *PNAS* 99:8078, 2002), have been shown to produce islets when

transplanted into the pancreas and to reverse insulin-dependent diabetes. Furthermore, Applicants have provided sufficient guidance in the present application for one of skill in the art to have a reasonable expectation that NSC, with a broad developmental capacity, would differentiate into insulin producing islets when transplanted into the pancreas. Thus, based on Applicants' specification, one of skill in the art would have a reasonable expectation of success in practicing the method of claims 18-20, a method of producing enhanced levels of insulin by implanting neural stem cells and/or progeny thereof into the pancreas.

The Examiner argued that while the pancreas itself supposedly provides a suitable environment for the differentiation of pancreatic progenitor cells into more differentiated pancreatic cells, "other tissues, such as the liver, will clearly not provide the necessary and proper factors." Thus, the Examiner alleged that the specification fails to provide adequate guidance for the practice of the claimed methods. See pp. 6-7 of the Office Action mailed August 13, 2002. Applicants respectfully disagree; however, the Examiner is requested to note that claims 18-20 are drawn to a method comprising implanting stem cells into the pancreas of a subject. As the Examiner acknowledged, stem cells implanted into the pancreas differentiate into pancreatic cells. Thus, the specification provides adequate guidance to practice the method of claims 18-20 with a reasonable expectation of success.

The Examiner argued that the specification presents no strategies for avoiding the acute graft rejection encountered with xenotransplantation (see p. 7-8 of Office Action). Applicants respectfully disagree. The specification teaches that when implanted stem cells are from donor tissue xenogeneic to the host various methods of immunosuppression can be used to reduce or

eliminate the immune response to the implanted tissue. Such methods include the use of immunosuppressive drugs, such as cyclosporin, or the use of locally applied immunosuppressants. As an alternative to immunosuppression techniques, gene replacement or gene knockout methods can be applied to stem cells, to eliminate the need to immunosuppress the recipient. See p. 10, lines 3-18 of the specification.

Further, Applicants submit that the methods of the claimed invention are not limited to xenogeneic transplantation. The specification teaches that stem cells can be obtained from the tissues of "a wide variety of animals, such as insects, fish, reptiles, birds, amphibians, mammals and the like. The preferred source is mammals, preferably rodents and primates, and most preferably mice and humans" (p. 8, lines 4-7 of the specification).

To overcome issues related to xenotransplantation, Applicants have isolated neural stem cells from the brains of embryonic mice genetically engineered to constitutively express the *Escherichia coli* gene LacZ (*Gtrosa-26* mice). These mice express bacterial ß-galactosidase in all cells and can therefore be readily distinguished from the host wild type cells. This information is presented in paragraphs 5-8 and Exhibits A and B of the Declaration under 37 C.F.R. § 1.132 of Pankaj Jay Pasricha and Maria-Adelaide Micci, a copy of which is enclosed herewith.

Briefly, staged-pregnant female *Gtrosa-26* mice at embryonic day 15 (E15) were used for the isolation of mouse NSC. The brains of embryonic mice were removed and the subventricular zone (SVZ) tissue dissected from each brain hemisphere. Single cell suspensions were made from this tissue using dispase/trypsin treatment and gentle trituration. The fractions were

combined, pelleted, and resuspended in Neurobasal medium containing B27, 2mM glutamine and penicillin-streptomycin. After 2-4 hours, the cells were spun down and media replaced with NB27 plus 20 ng/ml FGF and 20 ng/ml EGF. Under these conditions, embryonic NSC propagated in culture for several weeks retain their undifferentiated state. NSC isolated from E15 *Gtrosa-26* mice were plated onto poly-ornithine-coated glass slides and cultured in the absence of the mitogens EGF and FGF for 7 days. Nitric oxide (NO) production was measured and ionomycin was added at various time points to increase intracellular calcium (a required cofactor for nNOS). These results are shown in Exhibit A of the Declaration under 37 C.F.R. § 1.132 of Pankaj Jay Pasricha and Maria-Adelaide Micci. Exhibit A shows that *Gtrosa-26* mice NSC cultured *in vitro* express nNOS and produce NO. Differentiated *Gtrosa-26* mice NSC cultured for 7 days express nNOS as shown by immunofluorescent double-labeling for nNOS (green) and ß-tubulin(red) (A) and western blot analysis of CNS-NSC total proteins extract probed with a specific anti-nNOS antibody (B).

NSC isolated from E15 *Gtrosa-26* mice were transplanted into the pylorus of nNOS -/- mice. One week after transplant, cross sections through the pylorus were stained for ß-galactosidase. These results are shown in Exhibit B of the Declaration under 37 C.F.R. § 1.132 of Pankaj Jay Pasricha and Maria-Adelaide Micci. Exhibit B shows that NSC from *Gtrosa-26* mice can be tracked after transplantation into the gastric pyloris.

Applicant submits that one skilled in the art would conclude from the data in Exhibits A and B of the Declaration under 37 C.F.R. § 1.132 of Pankaj Jay Pasricha and Maria-Adelaide Micci that NSCs can be obtained from embryonic mice and are capable of differentiation into

neurons. Murine NSC express nNOS and produce NO when cultured *in vitro* and survive implantation into the gastric pylorus.

Applicants respectfully submit that the specification provides adequate guidance to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention of claims 18-20.

*New Claims 21-40*

Likewise, Applicants submit that the specification provides adequate guidance to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention of new claims 21-40. New claims 21-24 are drawn to methods of repopulating neurons, muscles, or other tissues within a gastrointestinal organ by implanting stem cells, progeny thereof, or combinations thereof into the gastrointestinal organ of the subject; new claims 35-37 are drawn to a method of providing a neurotransmitter within the gastrointestinal tract of a subject by implanting neural stem cells into the gastrointestinal tract of the subject; and new claims 38-40 are drawn to a method of producing nitrinergic neurons in a subject by implanting neural stem cells into the smooth muscle of the gastrointestinal tract of the subject.

Figures 1-3 of the specification show that NSCs develop into neurons that express nNOS and produce NO when cultured *in vitro*, while Figure 4 shows that NSCs implanted into the gastrointestional wall differentiate into nitrinergic neurons. Thus, Applicants submit that the specification provides adequate guidance to enable one skilled in the art to which it pertains, or

with which it is most nearly connected, to make and/or use the invention of claims 21-40 with a reasonable expectation of success and without undue experimentation.

*New Claim 37*

Applicants have demonstrated that a second neurotransmitter, substance P, is released from neural stem cells cultured *in vitro*. This data is presented as paragraphs 9-11 and Exhibit C of the Declaration under 37 C.F.R. § 1.132 of Pankaj Jay Pasricha and Maria-Adelaide Micci, submitted herewith.

Applicant measured intracellular calcium in human intestinal smooth muscle cells in co-culture with embryonic rat central nervous system derived neural stem cells in response to the nicotinic agonist DMPP. Cells were loaded with a calcium-sensitive fluorescent indicator and changes in intracellular calcium were measured in intestinal smooth muscle cells in response to application of the nicotinic agonist DMPP. To demonstrate that DMPP is acting on nicotinic receptor, the DMPP-induced response was blocked by the nicotinic receptor antagonist hexamethonium. To demonstrate that substance P released from CNS-NSC is responsible for the activation of smooth muscle cells, the DMPP-induced response was blocked by the substance P receptor antagonist CP-099994-01. The results are shown as Exhibit C of the Declaration under 37 C.F.R. § 1.132 of Pankaj Jay Pasricha and Maria-Adelaide Micci.

Exhibit C shows that DMPP induces an increase in intracellular calcium in intestinal smooth muscle cells when in co-culture with CNS-NSC (A). This effect is not observed when intestinal smooth muscle cells are cultured without rat CNS-NSC (B). The DMPP-induced

response in intestinal smooth muscle cells in co-culture with rat CNS-NSC was blocked by the nicotinic receptor antagonist hexamethonium (C), demonstrating that DMPP is acting on nicotinic receptor. (D) The DMPP-induced response in intestinal smooth muscle cells in co-culture with rat CNS-NSC was blocked by the substance P receptor antagonist CP-099994-01, demonstrating that substance P released from CNS-NSC is responsible for the activation of smooth muscle cells.

Applicants submit that one skilled in the art would conclude from the above data in Exhibit C that a substance P is released from rat CNS-NSC and that this substance P can activate adjacent intestinal smooth muscle cells. Thus, Applicants respectfully submit that the specification provides adequate guidance to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention of claim 37, to provide the neurotransmitter substance P within the gastrointestinal tract of a subject by implanting neural stem cells into a gastrointestinal organ of the subject.

*New Claims 41-43*

Claims 41-43 are drawn to a method of treating a disorder of the enteric nervous system in a subject by implanting neural stem cells into a gastrointestinal organ of the subject. In support of the claimed method of treating a disorder of the enteric nervous system, Applicants provide the information in paragraphs 12-14 and Exhibit D of the Declaration under 37 C.F.R. § 1.132 of Pankaj Jay Pasricha and Maria-Adelaide Micci, submitted herewith.

To determine the physiological effects of implanted neural stem cells, Applicants

measured the gastric emptying of liquids in nNOS-deficient mice after the implantation of rat NSC into the pyloric wall compared to the gastric emptying of liquids in control mice. nNOS -/- mice demonstrate abnormalities in gastric physiology and the gastric emptying of both solids and liquids is significantly delayed in nNOS -/- mice compared with control wild-type mice. The results are shown in Exhibit D of the Declaration under 37 C.F.R. § 1.132 of Pankaj Jay Pasricha and Maria-Adelaide Micci. Exhibit D shows gastric emptying of liquids at 20 minutes after gavage of dye in wild type mice, nNOS -/- mice, nNOS -/- mice 12 days after injection of PBS into the pylorus, nNOS -/- mice 12 days after transplantation of rat CNS-NSC into the pylorus.

Applicants submit that one skilled in the art would conclude from the above data in Exhibit D that liquid gastric emptying in nNOS -/- mice is improved after transplantation of rat CNS-NSC into the pyloric wall, showing that NSC transplantation is effective in correcting a neurotransmitter deficiency and producing a beneficial functional effect. Applicants respectfully submit that the specification provides adequate guidance to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention of claims 41-43, treating a disorder of the enteric nervous system by implanting neural stem cells into a gastrointestinal organ of the subject.

For the reasons discussed above, Applicants submit that the specification provides complete guidance to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention of claims 18-43. Withdrawal of this rejection under is respectfully requested.

## Summary

It is respectfully submitted that the pending claims 18-43 are in condition for allowance and notification to that effect is respectfully requested. The Examiner is invited to contact Applicants' Representatives, at the below-listed telephone number, if it is believed that prosecution of this application may be assisted thereby.

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CERTIFICATE UNDER 37 CFR §1.10:
"Express Mail" mailing label number: EV 183606853 US Date of Deposit: January 3, 2003
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**APPENDIX A - CLAIM AMENDMENTS**
**INCLUDING NOTATIONS TO INDICATE CHANGES MADE**
**Serial No.:**09/834,110
**Docket No.:** 265.00090101

Amendments to the following are indicated by underlining what has been added and bracketing what has been deleted. Additionally, all amendments have been shaded.

**In the Claims**

For convenience, all pending claims are shown below.

1. CANCEL

2. CANCEL

3. CANCEL

4. CANCEL

5. CANCEL

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11. CANCEL

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14. CANCEL

15. CANCEL

16. CANCEL

17. CANCEL

18. (AMENDED) The method of claim **[17]** **19** wherein the stem cells are selected from the group of multipotent, pluripotent, totipotent stem cells, and combinations thereof.

19. (AMENDED) **[The]** **A** method of **[claim 17]** **producing enhanced levels of insulin in a subject comprising implanting** **[wherein the cells are]** neural stem cells **and/or progeny thereof into the pancreas of the subject**.

20. (AMENDED) The method of claim **[17]** **19** wherein the cells thereof are derived from embryonic neural tissue, adult neural tissue, or combinations thereof.

21. (NEW) **A method of repopulating neurons, muscles, or other tissues within a gastrointestinal organ of a subject comprising implanting stem cells, progeny thereof, or combinations thereof into the gastrointestinal organ of the subject.**

22. (NEW) The method of claim 21 wherein the stem cells are selected from the group consisting of multipotent stem cells, pluripotent stem cells, totipotent stem cells, and combinations thereof.

23. (NEW) The method of claim 21 wherein the stem cells are neural stem cells.

24. (NEW) The method of claim 23 wherein the neural stem cells are derived from embryonic neural tissue, adult neural tissue, or combinations thereof.

25. (NEW) The method of claim 21 wherein the implanted cells repopulate neurons.

26. (NEW) The method of claim 25 wherein the subject suffers from a disorder of the enteric nervous system.

27. (NEW) The method of claim 26 wherein said disorder of the enteric nervous system is selected from the group consisting of achalasia, Hirschsprung's disease, congenital pyloric stenosis, reflux disease, irritable bowel syndrome, and intestinal pseudo-obstruction.

28. (NEW) The method of claim 21 wherein the gastrointestinal organ comprises a solid organ.

29. (NEW) The method of claim 28 wherein the solid gastrointestinal organ is the liver, the gall bladder, or the pancreas.

30. (NEW) The method of claim 21 wherein the gastrointestinal organ comprises a hollow organ.

31. (NEW) The method of claim 30 wherein the hollow gastrointestinal organ is the mouth, the esophagus, the stomach, or the bowels.

32. (NEW) The method of claim 21 wherein implanting cells into a gastrointestinal organ of a subject comprises administering the cells locally.

33. (NEW) The method of claim 32 wherein administering cells locally comprises injecting the cells into a wall of the gastrointestinal tract.

34. (NEW) A method of repopulating neurons within the gastrointestinal tract of a subject comprising implanting neural stem cells, progeny thereof, or combinations thereof into the gastrointestinal tract of the subject.

35. (NEW) A method of providing a neurotransmitter within the gastrointestinal tract of a subject comprising implanting neural stem cells, progeny thereof, or combinations thereof into the gastrointestinal tract of the subject.

36. (NEW) The method of claim 35 wherein the neurotransmitter is nitric oxide.

37. (NEW) The method of claim 35 wherein the neurotransmitter is substance P.

38. (NEW) A method of producing nitrinergic neurons in a subject comprising implanting neural stem cells into the smooth muscle of the gastrointestinal tract of the subject.

39. (NEW) The method of claim 38 wherein the neural stem cells are implanted into the pylorus.

40. (NEW) The method of claim 38 wherein the neural stem cells are implanted into the deuodenum.

41. (NEW) A method of treating a disorder of the enteric nervous system in a subject comprising implanting neural stem cells, progeny thereof, or combinations thereof into a gastrointestinal organ of the subject.

42. (NEW) The method of claim 41 wherein the implanted cells produce nitric oxide.

43. (NEW) The method of claim 41 wherein the cells are implanted into the pylorus.